

Dissipation Profile and Human Risk Assessment of Pyrimethanil Residues in Cucumbers and Strawberries

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Background. The present investigation was undertaken to study the persistence of pyrimethanil in strawberries and cucumbers following good agricultural practices under field conditions. A secondary objective was to validate a quick, easy, cheap, effective, rugged and safe (QuEChERS) method for the determination of pyrimethanil levels in strawberries and cucumbers using high performance liquid chromatography with photodiode array detection.

Methods. Pyrimethanil (20% wettable power (WP)) was sprayed on strawberries and cucumbers grown under field conditions at the dosage recommended by the manufacturer. The dissipation rates of pyrimethanil were described using first-order kinetics and its half-life was 2.9 days in strawberries and 2.2 days in cucumbers. A risk assessment was performed using the risk quotient (RQ).

Results. At fortification levels of 0.05, 0.1 and 0.5 mg/kg in strawberries and cucumbers, recoveries ranged from 90.1 to 109% with a relative standard deviation (RSD) ranging from 2.0-7.9%, which is within the acceptable limits for routine analysis of pyrimethanil residues. The limit of quantification (LOQ) was established at 0.05 mg/kg. The results showed that the RQ value was significantly lower than 1.

Conclusions. The results of the present study suggest that the risk of pyrimethanil use in strawberries and cucumbers at the recommended dosage is negligible to humans. This study could provide guidance for the safe and reasonable use of pyrimethanil in strawberries and cucumbers to prevent health problems in consumers.

Key Words. strawberry, cucumber, QuEChERS, pyrimethanil, residue, dissipation, Egypt

Competing Interests. The authors declare no competing financial interests.

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Introduction

Although some alternative pest management measures are available, effective crop protection most often depends on the use of synthetic pesticides.¹ Pesticides are widely used to protect crops from pest infestation; however, they may have a negative effect on the environment and the health of consumers.² Ideally, when applied to the plant, a pesticide should last for only as long as necessary to control harmful organisms and then decompose, leaving no residue in the harvested crop or at least none higher than the statutory maximum residue level (MRL) for the crop.³ The stability

of certain pesticides, and the fact that residues can remain in both processed and unprocessed foods, increases the human health hazard.⁴ Thus, pesticide analysis is an essential activity for food safety analysis. Analytical methods such as gas chromatography (GC) and high performance liquid chromatography (HPLC) have been accepted as standard techniques for pesticide residue analysis in agricultural samples. These analytical techniques generally require critical steps such as liquid-liquid solvent extraction and a series of cleanup procedures for sample preparation. Considering that the agricultural samples contaminated with pesticides must be routinely examined prior to being labeled table-ready foods, a simple method for rapid determination of

pesticide residues is essential.

In recent years, an increasing amount of interest has been focused on the development of novel, broad-spectrum fungicides with reduced risk to humans, non-target organisms, and environmental resources.⁵ Pyrimethanil [N-(4,6-dimethylpyrimidin-2-yl) aniline] is a product that is used to control a wide range of fungal diseases on fruits, vegetables and ornamental crops. It is a new anilino-pyrimidine foliar fungicide developed for the control of gray mold (*Botrytis cinerea*) on fruits and vegetables. Pyrimethanil is highly effective against all strains of *Botrytis* and has not shown cross-resistance to commercially available botrycides.⁶ To control residual

pyrimethanil levels in fruits and vegetables, it is necessary to establish reliable, efficient, and sensitive methods for monitoring pyrimethanil residues in different matrixes. Pyrimethanil at residue levels has been analyzed by GC equipped with selective and sensitive detectors in grapes, must, wine, processed plant tissue matrixes, soil, and vegetables.⁷ In addition, a manual of pesticide residue analysis has described a multi-residue method, which indicates that ethyl acetate and anhydrous sodium sulfate are able to extract pyrimethanil, among other pesticides, from fruits and vegetables.⁶ However, according to this methodology, only an ion trap detector (ITD) is able to identify pyrimethanil.⁸ An efficient analytical method for the determination of pyrimethanil is still lacking.

Cucumber (*Cucumis sativus*) is a widely cultivated plant in the gourd family *Cucurbitaceae*. It is a creeping vine that bears cylindrical fruits that are used as culinary vegetables. Cucumber is widely distributed in many parts of the world, and it is a very significant part of the diet in many countries. Pyrimethanil is considered to be an effective fungicide for controlling pests and diseases that affect cucumbers. The analyte has been registered in the guidelines for the safe use of pesticides in Egypt.

Strawberry (*Fragaria ananassa* Duch *cv. Sequoia*) is a popular crop that is widely grown worldwide. The commercial cultivation of strawberry requires frequent application of a large number of pesticides throughout the cropping season to control a variety of pests and diseases.

Regulatory work in Egypt has set pre-harvest intervals (PHIs) in relation to the pesticide MRLs—established jointly by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO)—aiming to prevent the distribution of unsuitable

Abbreviations			
ADI	Acceptable daily intake	MRL	Maximum residue level
ai/ha	Active ingredient per hectare	nm	Nanometer
bw	Body weight	PHIs	Pre-harvest intervals
CRL	Calculated residue level	PTFE	Polytetrafluoroethylene
EED	Estimated exposure dose	QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
FAO	Food and Agriculture Organization	RSD	Relative standard deviation
FI	Food intake	RTs	Retention times
g	Gram	RQ	Risk quotient
GEMS	Global Environment Monitoring System	S/N	Signal-to-noise
GC	Gas chromatography	v/v	Volume per volume
HPLC	High performance liquid chromatography	WHO	World Health Organization
ITD	Ion trap detector	WP	Wettable powder
LOD	Limit of detection	μL	Microliter
LOQ	Limit of quantification	μM	Micrometer

agricultural products that may exceed those MRLs.⁹ The PHIs investigate the amount of pesticide residues sprayed during the pre-harvest period and predict the amount of pesticide residues at the time of harvest by computing the biological half-life and the decay constant.¹⁰ The dissipation rate of synthetic chemicals after application depends heavily on several factors, including chemical and photochemical degradation, climatic conditions, volatilization, cultivated species, formulation class, and application mode.^{11,12} Thus, the dissipation curves reported in the literature are valid

only for a given crop under specific conditions.¹³ Therefore, it is essential to study the dissipation kinetics of pesticides in the environment, which could help improve our understanding of pesticide safety to humans, animals and the environment.

The present investigation was undertaken to study the persistence of pyrimethanil in strawberries and cucumbers following good agricultural practices under field conditions. A secondary objective was to validate a quick, easy, cheap, effective, rugged and safe (QuEChERS) type method

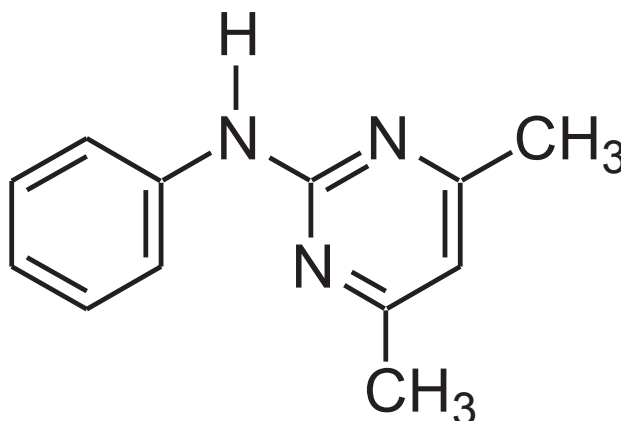


Figure 1 — Chemical structure of pyrimethanil

for the determination of pyrimethanil in strawberries and cucumbers using HPLC with photodiode array detection. Previous studies have shown the QuEChERS method to be the most effective, with a reasonably high rate of recovery and reproducibility.¹⁴ A field study was done to investigate the dissipation of pyrimethanil in strawberries and cucumbers. From the generated data, the pre-harvest residue limits were established based upon the dissipation pattern as well as the biological half-lives.

Methods

Chemicals and Reference Standard

Pyrimethanil standard (Figure 1) and formulation (20% wettable power (WP)) were donated by the Central Agricultural Pesticide Laboratory (Giza, Egypt). All organic solvents used in the study were of HPLC grade and purchased from Scharlau (Barcelona, Spain). Sodium chloride was of analytical grade, purchased from El Nasr Pharmaceutical Chemicals Company (Cairo, Egypt). Ultra-pure water was prepared by a millipore filtration system. The suitability of the solvents and other chemicals were ensured by running reagent blanks

before the actual analysis.

Field Experiment Design

Pyrimethanil (20% WP) was applied at the manufacturer's recommended rate in a water volume of 1000 liters per hectare using a knapsack sprayer fitted with a single-nozzle boom. To ensure the reliability of the experimental results, field management was carried out in accordance with local methods. There was no rainfall at any time during the experimental period.

Strawberries

The trials were conducted in a randomized block design in fields located in Ismailia governorate. Strawberries were planted in September 2013 at intervals of 0.25 m, in rows separated by a gap of 0.6 m. In March 2014, pyrimethanil was applied to separate plots measuring 40 m² in 3 replicates. None of the plots had been treated with pyrimethanil in the past. During the trial, the average minimum/maximum daily air temperature was 16/28°C. Samples were collected at random from sampling plots 2 hours after spraying, then 1, 3, 7, 10, and 14 days after pyrimethanil application. Immediately after picking, the samples were put into polyethylene bags and transported to the laboratory, where they were chopped

and thoroughly mixed. The samples were kept deep-frozen (-20°C) until analysis. Control samples were obtained from control plots.

Cucumber

Cucumbers were planted early January 2013 in rows of plants spaced 0.4 m apart, with 0.7 m between rows, in Ismailia, Egypt. Pyrimethanil was applied in late February 2013 to plots consisting of 2 rows, 15 m long. Plots were separated by a guard row of untreated cucumbers. Ripe fruits were taken for analysis 2 hours after spraying, then 1, 3, 7, 10 and 14 days after pyrimethanil application. During the trial, the average minimum/maximum daily air temperature was 13/25°C. Immediately after picking, the samples were put into polyethylene bags and transported to the laboratory, where they were chopped and thoroughly mixed. All the samples were stored at -20°C until further analysis.

Analytical Methods

Sample Extraction

A 10 g aliquot of chopped and homogenized samples was weighed in a 50 mL screw-capped tube; 5 g of sodium chloride and 20 mL of n-hexane/ethyl acetate (1:1 volume-to-volume (v/v)) mixture were added. The tube was vigorously shaken for 1 minute using a vortex mixer at maximum speed and shaken vigorously by hand up-and-down for 5 minutes. The extracts were centrifuged for 10 minutes at 3800 rpm and 4°C, the phase was allowed to separate, and 10 mL of the organic layer was removed, evaporated to dryness, diluted with 2 mL of methanol, filtered through a 0.22 µm polytetrafluoroethylene (PTFE) filter (Millipore, USA) and transferred into a glass vial for HPLC analysis.

HPLC Analysis

The HPLC analysis was performed

with an Agilent 1260 HPLC system (USA), with quaternary pump, autosampler injector, thermostat compartment for the column, and photodiode array detector. The chromatographic column was a Zorbax C18 XDB (250 mm x 4.6 mm, 5 μ m). The column was kept at room temperature. The flow rate of the mobile phase (methanol/water = 80:20 (v/v)) was 0.8 ml/min, and injection volume was 20 μ L. The wavelength for the detection of pyrimethanil was set at 265 nm. The residues in the real samples were tentatively identified by comparing the retention times (RTs) of the sample peaks with the RTs of the injected standards. Pyrimethanil was eluted at a retention time of 32.7 minutes. The chromatographic apparatus was controlled by Agilent's Chemstation software.

Validation Study

The method was subjected to a validation study before its application to determine the fungicide pyrimethanil residues in the samples. Recovery assays were carried out on samples of untreated strawberries, and cucumbers that were spiked with the target compound at 3 concentration levels in 5 replicates (Table 1). The method trueness and precision parameters in terms of average recovery and relative standard deviation were calculated and assessed according to the European Union guidelines (SANCO/12495/2011). The linearity of the chromatographic response was evaluated over a range between 0.01 and 2 mg/kg at 5 concentration levels.

Calculation

The dissipation kinetics of pyrimethanil residues were determined by plotting residue concentration against elapsed time after application and equations of best curve fit with maximum coefficients of determination (R^2) were determined.

Matrix	Fortified level (mg/kg) (n*=5)	Recovery (%)	RSD (%)
Strawberry	0.05	109	2.9
	0.1	90.1	7.9
	0.5	99.5	3.8
Cucumber	0.05	98.8	2.0
	0.1	100.9	2.7
	0.5	93.9	4.2

Table 1— Recoveries and Relative Standard Deviation (RSD) of Fortified Samples
*number of replicates

For dissipation of targeted fungicide in strawberries and cucumbers, exponential relationships were found to be applicable corresponding to the general first-order kinetics equation:

$$C_t = C_0 e^{-kt}$$

where C_t represents the concentration of the pesticide residue at the time of t , C_0 represents the initial deposits after application and k is the constant rate of pesticide dissipation per day. From this equation, the dissipation half-life periods ($t_{1/2} = \ln(2)/k$) of the studied fungicide were determined.

Dietary exposure calculation and risk assessment were calculated using the following equations:

$$\text{EED (mg/kg, bw)} = \text{CRL (mg/kg)} \times \text{FI (kg)} \div \text{bw (kg)}$$

$$\text{RQ} = \text{EED (mg/kg, bw)} \div \text{ADI (mg/kg, bw)}$$

where EED is the estimated exposure dose, bw is body weight, CRL is the

calculated residue level, FI is the food intake, RQ is the risk quotient and ADI is the acceptable daily intake. According to a summary report of the Global Environment Monitoring System (GEMS), the average standard strawberry intake of an adult is 2 g per day, while the average standard cucumber intake of an adult is 14.5 g per day.^{15,16} The average body weight of an adult is assumed to be 60 kg.¹⁷ The ADI for pyrimethanil is 0.2 mg/kg bw/day. An RQ value higher than 1 indicates that the risk of a pesticide for humans is unacceptable. By contrast, an RQ value less than 1 represents minimal risk to humans.¹⁸

Results and Discussion

Method Validation

Recoveries were determined at 3 fortification levels (Table 1). The mean recoveries from 5 replicates of the fortified strawberry and cucumber samples were in the range of 90.1-109%. The relative standard deviation (RSD) ranged from 2.0-7.9%, which is within the acceptable limits for routine analysis of pyrimethanil residues. Samples were quantified using external standards, with a linear working curve between 0.01 and 2.0 mg/L. The limit of

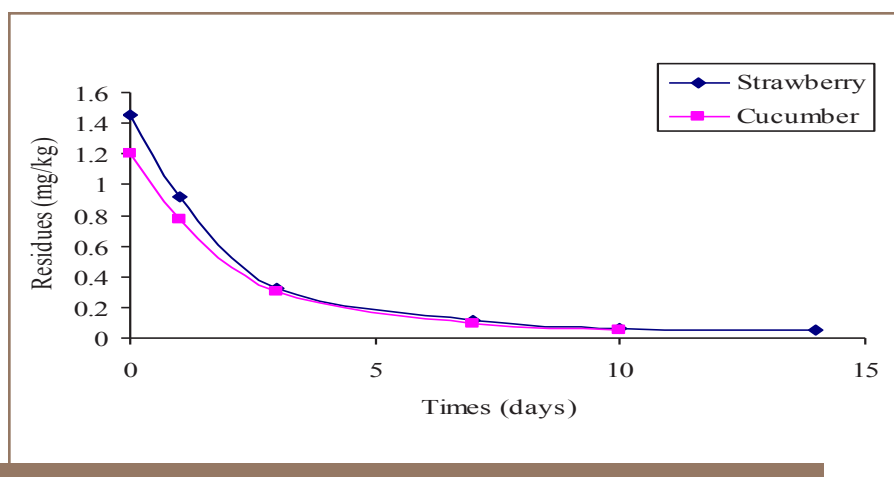


Figure 2 — Dissipation curve of pyrimethanil in strawberries and cucumbers under field conditions

Matrix	Regression equation	Correlation coefficient (r^2)	Half-life (days)
Strawberry	$C_t = 0.9534e^{-0.2385t}$	0.9005	2.90
Cucumber	$C_t = 1.0069e^{-0.3158t}$	0.9793	2.19

Table 2 — Regression Equation, Correlation Coefficient and Half-life of Pyrimethanil in Strawberries and Cucumbers

Days after application	Strawberry		Cucumber	
	Estimated exposure dose mg/kg bw/d	Risk quotient (RQ)	Estimated exposure dose mg/kg bw/d	Risk quotient (RQ)
0	0.0484	0.0242	0.0400	0.0200
1	0.0306	0.0153	0.0257	0.0128
3	0.0109	0.0054	0.0101	0.0050
7	0.0036	0.0018	0.0031	0.0015
10	0.0021	0.0010	0.0017	0.0008
14	0.0019	0.0009	—	—

Table 3 — Risk Assessment of Pyrimethanil Treated Strawberry and Cucumber

quantification (LOQ) was established at 0.05 mg/kg, which yielded a signal-to-noise (S/N) ratio of 10. The limit of detection (LOD) was 0.01 mg/kg.

Dissipation of Pyrimethanil Residue in/on Strawberries and Cucumbers

Figure 2 shows the dissipation curve of pyrimethanil in strawberries and cucumbers under field conditions. The initial residue level was much higher in strawberries than in cucumbers. A similar trend in results has been explained on the basis of the different morphologies of treated crops.^{19,20} The initial concentration in strawberries was 1.453 mg/kg with a half-life of 2.9 days, while in cucumbers the initial concentration was 1.201 mg/kg with a half-life of 2.2 days. As shown in Figure 2, there was a sharp decrease in the amount of pyrimethanil residues 3 days after application in strawberries and cucumbers. Concentrations were reduced to less than 8% by 10 days after application of pyrimethanil in both strawberries and cucumbers. Pesticide degradation in plants is governed by a number of factors, including its inherent chemical properties and the effect of other factors such as light, heat, pH, moisture, and growth dilution factor.²¹ The half-life ($t_{1/2}$), regression equation and correlation coefficient are summarized in Table 2.

The RQ value of pyrimethanil residues in/on strawberries and cucumbers was calculated by the equations shown in the Methods section. Results are presented in Table 3. The results showed that the RQ value of pyrimethanil in strawberries and cucumbers after application was significantly lower than 1. This suggests that the risk of pyrimethanil use in strawberries and cucumbers at the recommended dosage is negligible to humans.

Conclusions

The results of the present study may be of use to growers of strawberries and cucumbers in various parts of the world, particularly in climatic zones similar to that of Egypt.

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